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ABSTRACT

The pH of an acid ensilage of industrial fish (2% formic acid; 20°C) was raised with caustic soda to a pH of 11.0 five hours after ensiling began. In this manner, the enzymatic decomposition of the raw material was halted. The high pH caused intact protein to gel and thereby resulted in ensilage with high texture. This basic ensilage could be used as the basis for fish feeds containing low amounts of binding agents and resulted in a feed with low water solubility and acceptable pelleting properties. The

composition of the feed was as follows: 55% basic ensilage, 43% capelin meal, 1% guar gum and 1% vitamin meal. Feed trials with fish in 4 test groups gave clear indications that this feed produced growth comparable to dry pellets. Feed factors in all test groups were low.

3 KEY WORDS

FISH FEED

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TECHNOLOGICAL PROPERTIES OF FISH ENSILAGE

BY

KRISTIAN PRYTZ AND EVEN STENBERG

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1. SUMMARY

pH in an acid ensilage of industrial fish (2% formic acid; 20°C) was raised with caustic soda to a pH of 11.0 five hours after ensiling began. In this manner, the enzymatic decomposition of the raw material was halted. The high pH caused intact protein to gel, and thereby resulted in ensilage with high texture. This basic ensilage could be used as the basis for feeds containing low amounts of binding agents, and resulted in a feed with low water solubility and acceptable pelleting properties. The composition of the feed was as follows:

55% basic ensilage
43% capelin meal
1% guar gum
1% vitamin meal

Feed trials with fish in 4 test groups gave clear indications that this feed produced growth comparable to dry pellets. Feed factors in all test groups were low.

2. OBJECTIVES

The objectives of the project were to develop an alternative to the traditional ensilage of industrial fish,

which could constitute the basis for a feed with no content or a low content of polysaccharides.

3. PROJECT BACKGROUND

The production of cultivated fish in Norway will in 1987 amount to between 50,000 and 60,000 t. The growth of the aqua culture industry has been great and production has increased from 4,000 t in 1979 to 46,000 t in 1986. The prognosis for 1988 indicates a product volume of 74,000 t. (1). There is much evidence that there will be a need in the future for new, cheaper and not least, nutritionally correct sources of feed for farmed fish.

Ten years ago, there was some interest in fish ensilage as feed for salmon in Norway. However, due to insufficient quality control, many fish farmers had bad experiences with this feed, and the interest decreased. The dominant feeds for today's farming of salmon are dry pellets and frozen capelin. The dry feed is practical and easy to use, with the addition of vitamins necessary for the growth and well-being of the fish. The feeding systems can be simplified and the feed is easier to use than feed based on fish waste/industrial fish. In the last few years, however, questions have been raised regarding the solubility of the dry feed in the intestines of fish and its high content of polysac-

charides. Producers have emphasized that the polysaccharides have been made digestible and will constitute part of the energy of the feed. However, fish cannot regulate their metabolism of sugar in the same way as more advanced animals. The insulin production of fish is low, and a surplus of sugar added through the feed is channelled to the liver of the fish. This results in liver damage when high doses are given for long periods. When frozen capelin is used, it is ground and mixed with binding agents. But this produces a sticky feed, with high water solubility, not very suitable for automatic feeding. The feed is a source of contamination and may contribute to microbial anaerobic growth below the fish farm, resulting in poisoning.

The digestion of cultivated fish is very low during the cold season. Bleeding anus is a phenomenon observed in cultivated fish, particularly in the winter. There are many signs indicating that the dry feed is not fully dissolved during digestion, and that the hard consistency of the feed can result in damage to the intestinal epithelium of the fish and the bleeding anuses that have been observed. Small tears in the intestinal epithelium and a bleeding anus can be crucial to the health of the fish when they are living in an otherwise stressful situation, and could possibly be a contributing factor in cases of disease.

The above-mentioned properties of the dry feed may stimulate a renewed interest in industrial fish, fish waste and fish cut-offs as feed for cultivated fish, provided that some of the weak points of this type of feed can be improved. Industrial fish appears logical today as a more "biologically" correct feed for farmed salmon and trout, because it is one of the naturally available sources of food for the fish. Use of industrial fish as fish farm feed should also be seen in the context of a steadily growing interest in the farming of white fish. Even higher demands will most likely be made on the composition of the feed for this purpose. For example, feed for white fish must have a protein content that is higher than the fat content, and it must contain less sugar than feed intended for salmon. The use of correct feed for cultivated fish ought to result in increased well-being and improved health among the fish.

In addition to the use of frozen industrial fish, as described above, industrial fish and fish waste were previously used as fish feed after ensiling with acid. However, in acid ensiling, acid proteolitic enzymes split the proteins into peptides and amino acids, and the ensilage loses its consistency and becomes very liquid. This necessitates the addition of a large amount of binding agents that contain polysaccharides, in order to give the feed the right consistency and to make the feed easy to handle, with

low water solubility and little waste of feed. Consequently, the feed will contain a relatively high amount of polysaccarides.

In the project "Technological Properties of Fish Ensilage", we have attempted to ensile industrial fish while preserving the consistency characteristics of the ensilage, so that this ensilage can be used as fish farm feed without having to add considerable amounts of polysaccharides.

4. INTRODUCTION

Until a few years ago, capelin represented a considerable resource for Norway. The capelin stock is at the moment small, but is expected to increase. The greater part of the capelin catch is used for meal and oil production, but from a nutritional view-point, capelin should also be suitable as feed for farmed fish. However, the use of industrial fish for this purpose requires some quality control. It requires that fresh raw material is treated in such a way that a satisfactory feed can be produced. The raw material can be frozen before the decomposition processes have gone too far, but an alternative is to conserve it through ensiling. The ensilage could be produced with capelin alone, or mixed with fish waste, fish cut-offs, or

catches of other species from, for example, shrimp trawling, which cannot be used for other purposes.

Methods of ensiling fish

Ensiling is a term borrowed from agriculture where fodder grass is preserved by adding an organic acid in a silo. Industrial fish and fish waste can be preserved in the same manner by lowering the pH with an organic or inorganic acid, or through lactic acid fermentation (2). In the two former cases, almost all microbial activity in the fish ceases, while in the latter case, the lactic acid bacteria will dominate the development of bacteria and prevent rotting through lactic acid fermentation. There are also examples of attempts made at preserving fish waste by increasing the pH with ammonia to a pH range where all microbial activity ceases.

Microbial activity is most easily prevented by ensiling with an organic acid. The cell membranes of micro-organisms are non-permeable to H^+ ions, and an organism can thereby survive in somewhat acidic conditions, because it maintains an environment that is intracellularly neutral. In ensiling with organic acids with a pK-value higher than the pH of the ensilage, a portion of the organic acids will be present in an undissociated form. In this form, both formic acid and

propionic acid will diffuse through the cell membrane and dissociate there due to the neutral intracellular environment. H+ will be liberated intracellularly, with a devastating effect on the micro-organism. During ensiling at a pH below 3.75, at least 50% of the formic acid and at least 50% of the propionic acid will be present in an undissociated form, because their pK-values are 3.75 and 4.86, respectively (2). Earlier ensilage trials showed good preservation with the use of a mixture of formic acid and propionic acid, where propionic acid, in particular, prevented growth of fungi on surfaces (2). Feed trials where this ensilage was used as the basis for the fish farm feed have, however, shown that salmon had a tendency to reject feed containing propionic acid (1). In those cases where acid ensilage is used in feed today, the only organic acid used is formic acid.

Organic acids have a better anti-microbial effect when used during ensiling than do inorganic acids. An ensilage based on ensiling with organic acids does not have to be neutralized before adding binding agents. But inorganic acids are cheaper than organic acids. In cases where fish cut-offs containing bones are mixed in, it may be economically desirable to ensile with a mixture of inorganic and organic acids. pH will then be brought down into the acid range with a cheap inorganic acid, and the anti-microbial

effect is ensured by adding smaller amounts of organic acid. This type of ensilage is used successfully with a mixture of formic acid and sulphuric acid.

One of the reasons that fish keep less well than warm-blooded animals is their low content of glycogen. Post mortem, glycogen is split via sugar into lactic acid. The final pH, due to production of lactic acid, is thereby lower in warm-blooded animals after slaughtering and hanging than the final pH in fish. By increasing the production of lactic acid, the fish will keep better. This can be achieved by adding lactic acid bacteria and by making their conditions for growth more favourable, so that the fish is preserved through lactic acid fermentation.

Preservation with the aid of lactic acid bacteria has long been a familiar process in the dairy industry. These bacteria are here used, for example, in the production of yoghurt. When preserving fish with lactic acid bacteria, the growth environment of these bacteria must be favoured. The natural microflora of fish also contains few lactic acid bacteria. It is therefore necessary to add a starter culture to initiate fermentation. Lactic acid bacteria are dependent on free sugar for growth (glucose, fructose, ribose). Fish contain little free sugar, but large amounts of free amino acids. The latter constitute the substrate for the common

decomposition bacteria in fish. In order to initiate a successful lactic acid fermentation, it will be necessary to mix ground/chopped fish with sugar and a starter culture. There have been trials with cheap sugar sources, for example molasses, but it was reported that large quantities (up to 10%) are needed in the mixture (2). In the initial stage during preservation, while pH is still neutral, both decomposition bacteria and lactic acid bacteria will grow. As the organisms gradually produce lactic acid, pH will fall below 4.0, and the lactic acid bacteria will become the predominant organisms in the mixture. It is of crucial importance to secure the growth of homofermentative lactic acid bacteria. These produce primarily lactic acid free from sugar. The heterofermentative bacteria produce, besides lactic acid, considerable amounts of acetic acid and CO_2, and this leads to less effective preservation. The gas production can also create unsuitable increases in volume during fermentation in large tanks. However, in the pH range below 4.0, homofermentative Lactobacilli are selected (1).

The preservative effect after a successful lactic acid fermentation is mainly attributable to the production of lactic acid and the resulting low pH. But it has also been claimed that individual species of lactic acid bacteria produce substances that have an antibiotic effect (3,4). The result is a hygienically acceptable product and that

coliform bacteria, typhoid bacteria and coagulase positive staphylococci are destroyed during ensiling (5,6). It has further been claimed that Clostridium spores are destroyed (6). Fermentation of fish with lactic acid bacteria, however, is a process that requires much expertise and good raw material. The chances of uncontrolled fermentation are great, and this could have considerable economic consequences for large scale industrial production. The content of ammonia in an ensilage conserved with lactic acid bacteria also appears to ge greater than in a traditional acid ensilage. This is partly attributed to the growth of decomposition bacteria in the initial stage of fermentation, before the pH falls.

Biochemistry of fish ensiling

When ensiling fish with acid, pH is brought down to 3.0-4.0 and the microbial activity is inhibited. But the biochemical reactions suited to acid conditions continue in a process called autolysis. This means that enzymes split bonds in the raw material, and cause disintegration of tissue. The raw material decomposes to a certain degree.

The overwhelming part of the enzymatic activity in fish raw material at a pH of 3.0-4.0 is attributed to proteolytic enzymes. These are mainly found in the digestive system of

the fish, which leads to the situation where the autolytic processes in an ensilage based on the highest quality fish cut-offs occur much later than the autolytic processes in an ensilage based on raw material where the digestive system is also included. The acid proteolytic enzymes, the pepsins, are present in several forms in, for example, cod entrails. We here distinguish between pepsin I and pepsin II, where pepsin II is responsible for 80% of the total activity (7). There are two variants of pepsin II (a and b) with a minor difference in their isoelectric point (pI 4.2 and 4.5 respectively). There is also a small difference between the molecular weight of pepsin I (MW 35,500) and pepsin II (34,000), but pepsin I has a considerably higher isoelectric point (pI 6.9) (8).

The acid proteolytic enzymes cause the protein in the raw material to split into peptides and free amino acids. Enzyme activity is at its maximum at a pH of between 2 and 4, in the normal pH range for ensiling, if the activity is measured with haemoglobin as the substrate. But high activity can also be observed at higher pH values if a different substrate is chosen (e.g. casein). However, in the pH range down towards a pH of 2 and lower, the activity of pepsin I is reduced, while the activity of pepsin II is maintained. Above a pH of 6.0, the enzymes quickly become inactive. The autolytic processes depend not only on enzyme

content and pH. The temperature is crucial for the speed of the ensiling process, and thereby for how fast the fish raw material will decompose. The optimum temperature for the pepsins is at 40°C, but they are immediately inactivated when the temperature exceeds 45-50°C (7). The ensiling process can thereby be controlled to a certain degree by varying the temperature, the pH, or both of these parameters.

The presence of autolytic processes can be seen in the decomposition of the raw material. The rate of decomposition will depend on the temperature, but increases dramatically at around 20°C. This can be attributed to a denaturation of the structure of connective tissues, which increases the effect of the pepsins (9). Ground industrial fish is then changed from a consistency of minced fish into a liquid form. When put aside with no stirring, fat is separated and deposited at the top of the ensilage. There will always be a sediment of undissolvable material, which can be attributed to protein with amino acid sequences which are hardly attacked by pepsins (2).

The characteristic of acid ensilage is that it is microbially stable and that protein is split into smaller peptides or free amino acids. Most amino acids are stable in an acid ensilage (2). However, a decline in the content of

tryptophan in ensilage with formic acid at 20°C (pH 4) has been observed, but this decline was considerably reduced at low temperatures. It is also questionable whether histidine and methionine are stable (10,11). Histidine may be a limiting amino acid, dependent on the quality of the raw material. Histidine is quickly degraded by decomposition bacteria at a neutral pH. In raw material of poor quality, this amino acid may have been destroyed before ensiling starts. When using acid ensilage for fish farm feeds, too much splitting of the proteins is not desirable. This results in the formation of bitter peptides, which can reduce the appetite of the fish for the feed (12).

Ensilage as feed for farmed fish

As previously mentioned, acid ensiling of industrial fish and fish waste will cause the ground raw material to lose its firm consistency and it will become very liquid. When ensilage is used for animal feed, this situation must be compensated for by mixing in considerable quantities of binding agents containing polysaccharides, which will give the ensilage the correct consistency for producing pellets. An attempt has been made to reduce these undesirable amounts of binding agents either by subsequent treatment of the acid ensilage, or by modifying the conditions during ensiling, e.g through the use of lactic acid bacteria.

Bjugn Industries Inc. has produced ensilage for fish feed, where an attempt was made to reduce the need of binding agents through heat treatment and concentration of the ensilage after ensiling (12). Fresh raw material was ground and mixed with formic acid to a pH of 4. The paste would then be microbially stable. The temperature was then kept around 30°C until the protein was partially split by enzymes. In order to prevent complete splitting and formation of bitter peptides, the enzymatic activity was stopped by brief pasteurization. The pasteurization time was short and it was reported that it did not have an effect on the quality of the protein. The pasteurized paste was then concentrated at low temperature in a vacuum, and a paste consisting of 50% dry matter was obtained. Fish farm feed made from unconcentrated ensilage contains up to 50% binding agents, concentrated ensilage in feed results in a need for only 10-15% binding agents.

Ensiling where the consistency is preserved has also been tried by Biotec Inc. of Tromsø through lactic acid fermentation of industrial fish and fish waste (13). This method consists of grinding the raw material and heating it with the aid of two heat exchangers to 60°C and 90°C, respectively. In this way the enzymatic activity is stopped. The desired amount of fat can be separated and small amounts of formic acid, antioxidant and molasses are then added to

the paste. The temperature is reduced to 20-25°C, and a culture of lactic acid bacteria and a dry matter compound are added to the mixture. The finished product, called Ensiferm, has a porridge-like consistency and is reported to have a fresh, tart smell. Ensiferm will require an addition of 15-20% binding agents. In comparison, acid ensilage requires about 50% binding agents. There are presently no producers who ensile industrial fish in this manner, due to the relatively high capital investment costs.

Any use of industrial fish and fish waste for fish farm feed will require that the feed is supplemented with vitamins. Fish contain the enzyme thiaminase, which causes thiamine (vitamin B_1) to be rapidly broken down after the catch. Thiaminase is destroyed when heated to 82°C (14) in neutral conditions, and the degradation of vitamins can in this way be prevented. However, thiaminase is more stable in acid conditions and it has not been demonstrated that the enzyme is inactivated by heating of acid ensilage, but it is still unlikely that it could withstand boiling (2). Another of the vitamins, ascorbic acid (vitamin C), is also unstable and is degraded when fish feed is stored. For these reasons, it is common to add a vitamin mix to ensilage immediately before pelleting.

5. MATERIALS AND METHODS

Raw material

In the ensiling trials, summer capelin caught at Jan Mayen during the 1986 season was used. The raw material contained approximately 20% fat and 14% protein, and was frozen in 20 kg blocks within 24 hours after catch. A large amount of bait was observed in the fish. The same raw material was used for laboratory trials and pilot trials.

Ensiling conditions

For the laboratory trials, frozen raw material was defrosted overnight, weighed and ground for a maximum of 2 minutes in a high-speed grinder (Robot Coupe S.A., Model R40B) together with 2% (w/w) formic acid (85% technical quality). The homogeneous paste was transferred to 4 measuring cylinders of 4 L each and mixed (120 rpm, 20^{o}C). The mixer was manufactured at the Research Institute for Fisheries Technology and is shown in Figure 1. pH was adjusted to basic range with a saturated solution of caustic soda (NaOH). This ensilage will be referred to as basic ensilage in the rest of this text. Basic ensilage was compared to ensilage produced as described above, but where

the pH was not adjusted upward (acid ensilage). No antioxidant was added in these trials.

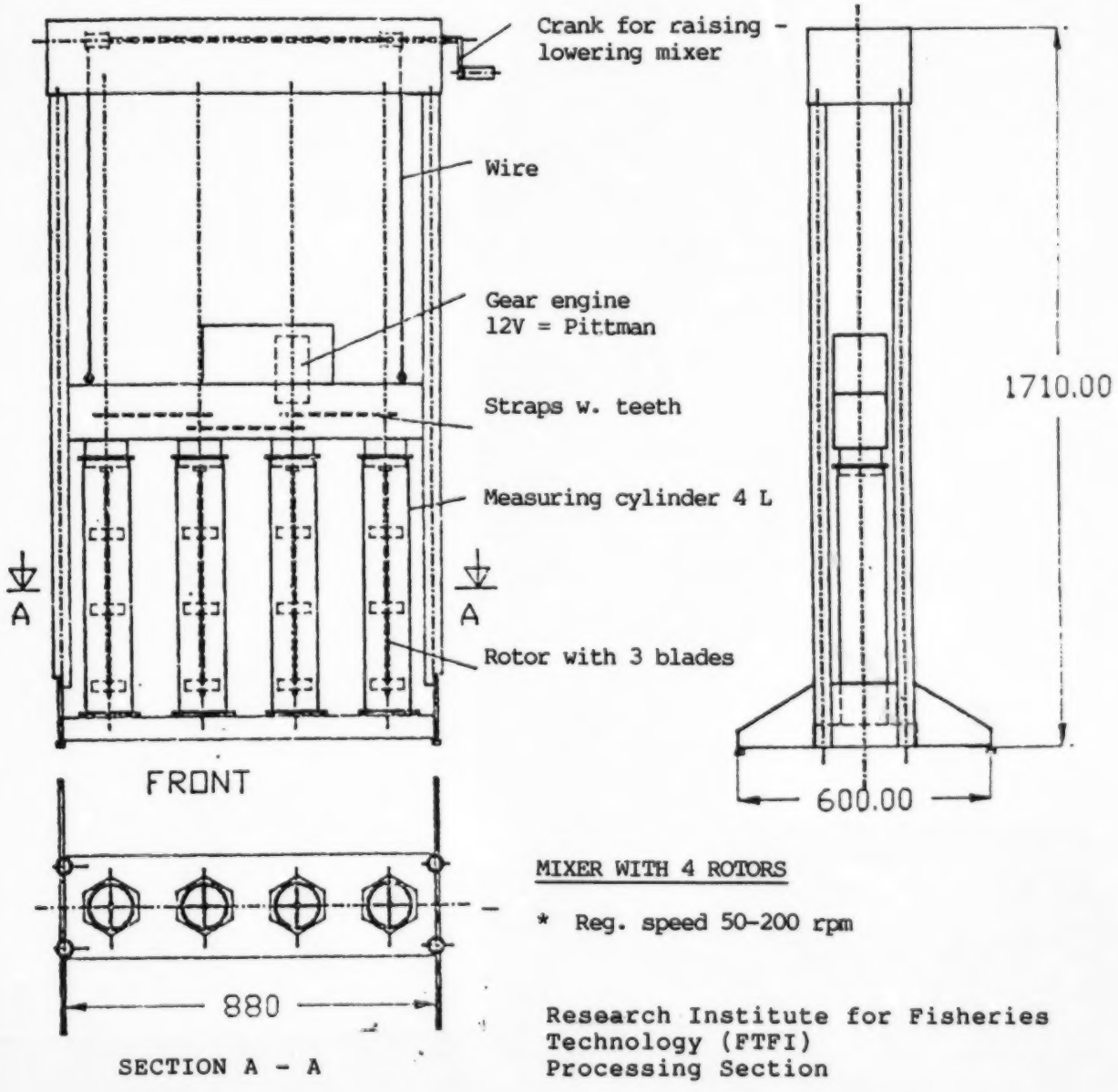

Figure 1. The figure shows mixer for laboratory scale ensiling.

* Transl. note: Unreadable word due to poor photocopy

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In the pilot trials, 400 kg of raw material was ground in a high-speed grinder (Kramer Grebe GmbH & Co., type VSM 65). The container with ground paste was emptied into a 1,200 L cubic tank equipped with a mixer (of make Scaba). Two percent (w/w) formic acid (85% technical quality) was added. Due to the viscosity of the paste, the acid was mixed in by using a pump to circulate it through the tank (Netzsch Monopumpe). pH was adjusted to 10.8 with a saturated solution of caustic soda after 20 hours of acid treatment. The temperature was 2°C when the acid was added, and 20°C when the soda was added. After the soda had been mixed in, the ensilage was poured into a container and placed in cold storage (4°C). Two hundred ppm of ethoxyquine[1] were added to the ensilage as an antioxidant. This ensilage was used as the base for preparing fish farm feed for the feed trials.

Viscosity measurements

The viscosity of the ensilage was measured with a Brookfield viscosity meter (Brookfield Engineering Laboratories Inc.). No linear correlation was found between spindle speed and viscosity meter reading. The trials were therefore performed with the same spindle speed (20 rpm), while the spindle was altered depending on the viscosity range that

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[1] Transl. note: Norwegian "ethoxquin", a compound not found in available English chemical dictionaries.

was required. All viscosity measurements were done in a 600 ml glass beaker with the same quantity of ensilage.

Measuring proteolytic activity

The activity of acid and basic proteinases was measured according to a method described by Barrett (1972). Haemoglobin was used as substrate at a pH of 3.0, and casein was used as substrate at a pH of 9.0.

Amino acid analyses

To both acid and basic ensilage (each corresponding to 30 mg of protein) 6N hydrochloric acid (2 ml) was added in hydrolysis tubes. The samples were evacuated and the hydrolysis tubes were sealed. The ensilage was hydrolyzed at 120^{o}C for 24 hours. After completed hydrolysis, material that could not be hydrolyzed was removed by filtering. The hydrolysate was evaporated in a rotary evaporator and dissolved in a citrate buffer (pH 2.2) until a concentration of amino acids of 0.2 mg protein/ml was obtained. The amino acid analysis was performed with a high pressure liquid chromatograph Spectra Physics 8700xR extended range LC pump, a Spectra Physics 8400 UV/Vis detector, and a Spectra Physics 4700 integrator. The amino acids were separated on a Supelcosil LC18 column (250x4.6 mm) with mobile phase A

(0.14M NaAc, pH 6.4, 0.05% TEA, 6% MeCN) and B (60% MeCN). The gradient was 5-80% B (0-20 min) and 80-100% B (20-30 min). The elution speed was 1.0 ml/min and the amino acids were detected at 254 nm.

Analysis of hydroxyproline

L-hydroxyproline was determined according to a method described by Leach (1960) (15).

Measurement of peroxide number

The peroxide number was determined according to Wheeler's method (16). Fat was separated through ether extraction, the extract was filtered and the ether evaporated. The sample (2 g) was dissolved in 35 ml of chloroform: acetic acid (2:3) with addition of Na_2CO_3 in excess and KI (1 ml). The components were mixed and distilled water (100 ml) was added after one minute. The solution was titrated with $Na_2S_2O_3$ until the colour changed using 0.2% starch as the indicator. The P-number was recorded in mmol of peroxide/1,000 g of fat.

Preparation of trial feed

For preparation of trial feed for testing of consistency and solubility, basic or acid ensilage was mixed with Salmonix 10, Salmonix 45, guar gum and Protatek[1] BF 120 IMP, all delivered by Skretting Inc. In addition, xanthan (Kelco Inc., USA), Drinde 1015F (Fjordimport, Haugesund) and capelin meal (NSM from Nordsildmel Inc.) were studied for their consistency-affecting properties. No antioxidants or vitamins were added to the trial feed. However, for the feed trials, 200 ppm of ethoxyquine was added to the feed as an antioxidant as well as a mixture of vitamins (Skretting Inc.)

Consistency measurements

After preparation of the fish farm feed, mixed with different types and amounts of consistency agents, it was necessary to measure the consistency of the finished feed. For the measurements, a texture press was used with a 100 kg weighing cell (20; Figure 2). A 50 ml disposable syringe was filled with 30 ml of feed. A hole with a diameter of 8 mm had been drilled at the end of the syringe. The effect necessary to push the syringe plunger down 20 mm at a speed

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[1] Transl. note: 'Protatec' and 'Drinde' appear to be product names.

of 5 mm/sec., and thereby push a specific amount of feed out through the hole, was determined with the aid of the texture press. The effect was determined on the basis of the mean of 3 tests. The effect required showed an exact correlation with the consistency of the feed based on sensory evaluation.

Solubility measurements

In order to gather information about the solubility of the feed, the feed was pelleted using pilot scale pelleting equipment. Pellets (10) with a 10 mm diameter and a length of 12 mm were weighed and dropped into 800 ml of distilled, constantly circulating water. A 1000 ml glass beaker and a propeller mixer, placed about 75 mm from the bottom of the glass beaker, with a speed of 180 rpm, were used. The water temperature was kept at a constant 20°C. Water samples were removed after 4 minutes and the amount of dry matter separated from the pellets into the aqueous phase was measured. The solubility properties of the ensilage feed were in this manner compared to the solubility properties of commercial dry feed.

Feed for feed trials

The feed trials were performed with feed based on a basic ensilage, as described in "Ensiling conditions" (pilot scale). The feed had the following composition:

Basic ensilage	55%
Capelin meal (NSM)	43%
Vitamin meal	1%
Guar gum	1%

Basic ensilage was mixed with the other ingredients in a blender and pelleted in a pilot scale pelleting unit. Pellets were made daily, with a diameter of 6 mm and a length of 12 mm. Due to unforeseen technical problems with the pelleting unit, the capelin meal was replaced with Salmonix 45 during the first four weeks of feeding.

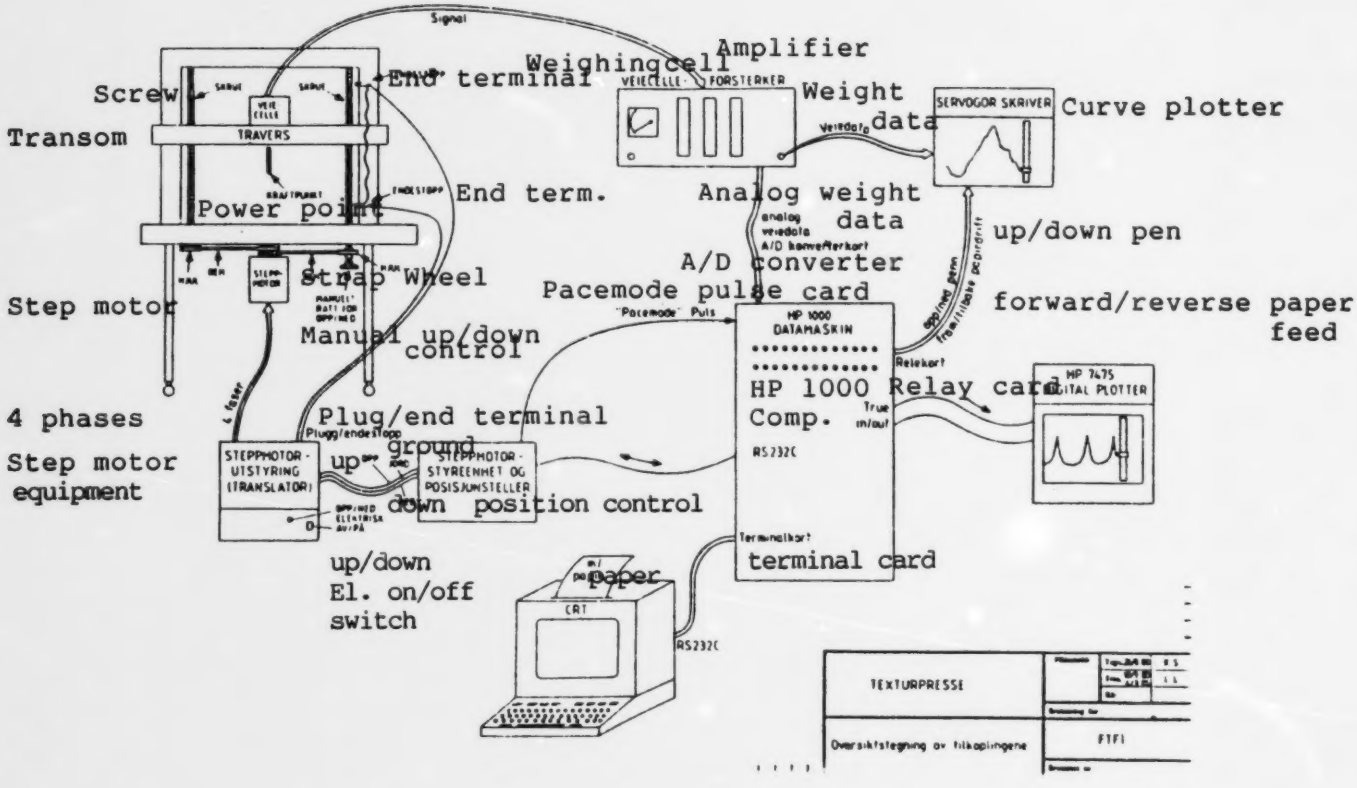

Figure 2. The figure shows the texture press arrangement for consistency measurements.

Chemical composition of feed

Protein in the produced feed was determined with Kjeldahl's method. The fat content was determined according to a method described by Bligh & Dyer (1959) (17). The water content was determined through weight change after evaporation at 105°C for 12 hours, and the ash content after combustion at 540°C. The quantity of carbohydrates in the feed was calculated according to the dry matter, with corrections for protein, fat and ash content.

Trial fish and feeding

The feed trials were begun in June, 1987, with around 400 fish of a size around 220 gram each. The fish were separated into 4 nets[1] and around 200 were fed with feed based on basic ensilage and around 200 were fed with dry feed (Tess Edel). Feeding was carried out manually twice daily. The fish were fed until they stopped eating. The amount of feed given was recorded. Growth was determined on 20% of the trial fish by anaesthetizing (chlorbutanol;0.1%, w/w), weighing and measuring them.

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[1] Transl. note: Norwegian 'maerer' refers to nets with bottoms that can be closed and opened.

Statistical methods

The difference in growth between the trial groups was analysed through analysis of variance. A statistics program was used for the analyses (Statpro, Penton Software Inc.).

6. RESULTS AND DISCUSSION

Ensiling

During regular acid ensiling of industrial fish, as described in the section "Materials and methods", the autolytic processes are initiated, and protein is split into peptides and free amino acids. Cell tissue starts dissolving, fat is separated and the consistency of the paste becomes very liquid. The change in consistency can be illustrated by measuring the viscosity as a function of the time elapsed since ensiling began. This is shown in Figure 3 for ensiling at 20°C.

The results show that the viscosity was reduced by half already after 3 hours when ensiling at this temperature, and that the ensilage is very liquid already 24 hours after the start of ensiling. Moreover, the viscosity measurements appear to be an acceptable measurement of the consistency within the range.

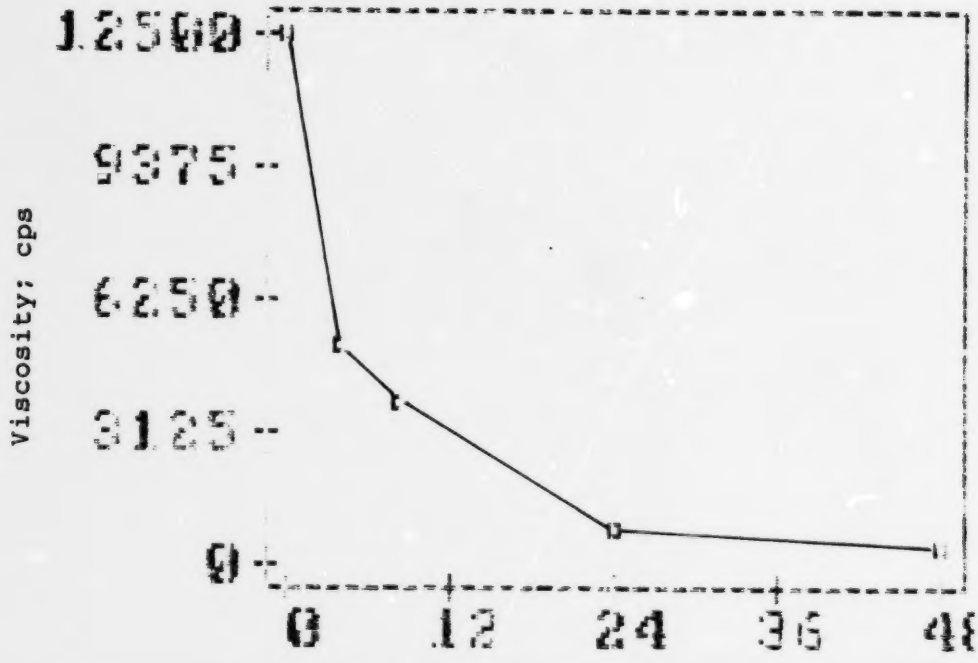

Figure 3. The figure shows viscosity (cps) as a function of time (hours) elapsed from start of ensiling for an ensilage of ground capelin with 2% formic acid (w/w) at 20°C.

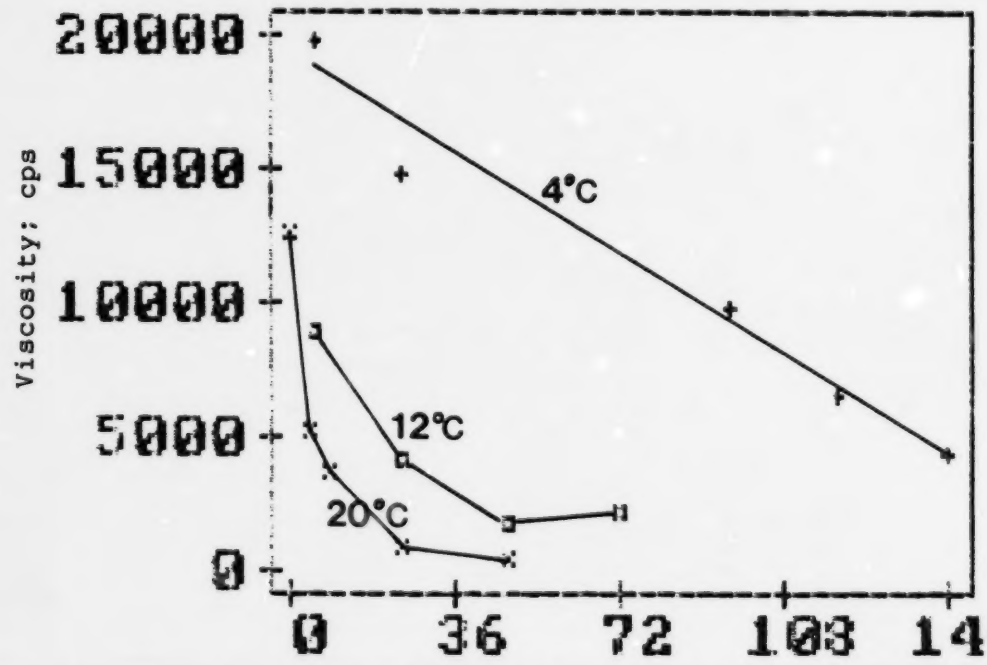

Figure 4. The figure shows viscosity (cps) as a function of the time (hours) after start of ensiling for an ensilage of capelin with 2% (w/w) formic acid at various temperatures.

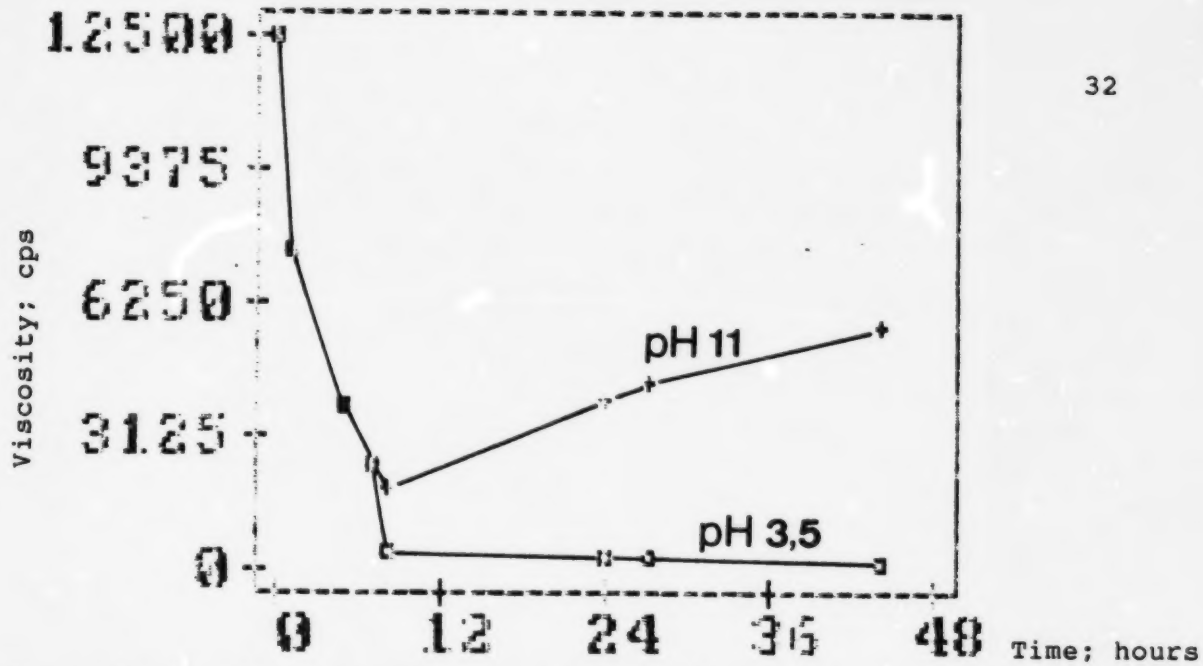

Figure 5. The figure shows viscosity (cps) as a function of time (hours) when capelin is ensiled with formic acid (2%, w/w; 20°C) and pH adjusted to 11 (caustic soda) seven hours after start of ensiling.

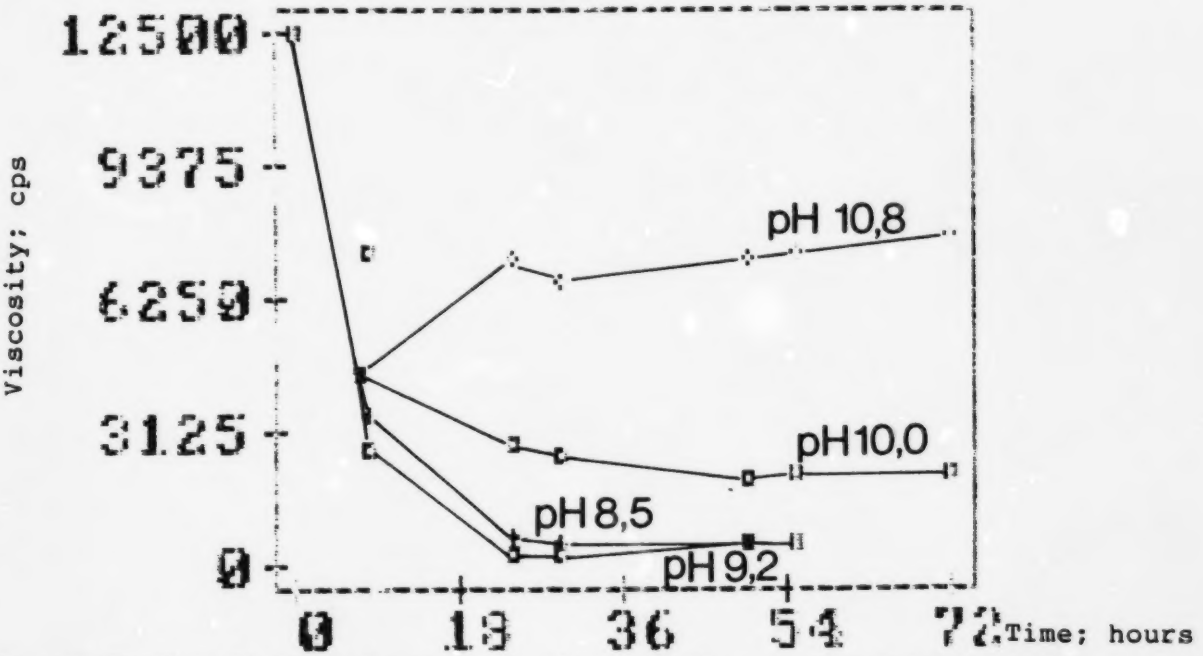

Figure 6. The figure shows viscosity (cps) as a function of time (hours) when pH in an acid ensilage of capelin (2% formic acid; 20°C) is adjusted upward to various pH values (caustic soda) 5 hours after start of ensiling.

The speed of the autolytic processes, and thereby the protein splitting of the acid proteinases, will vary considerably with the temperature. Figure 4 shows the changes in viscosity of an acid ensilage as a function of the time after ensiling at different ensiling temperatures. In cases when the viscosity decrease, and thereby the decomposition of protein chains is to be stopped at a desired level, it is of crucial importance to know at what temperature the ensiling took place. At a low ensiling temperature, viscosity will decline considerably more slowly than when ensiling takes place at 20°C.

The low viscosity of an acid ensilage of industrial fish will, to a great extent, contribute to a large requirement of binding agents, when this ensilage is to be used for fish farm feed. Attempts were previously made to stop the development of such a liquid consistency by heat treating the ensilage before the decomposition of proteins was complete and bitter peptides formed. This pasteurization step was carried out with regular acid ensilage and before ensiling with lactic acid bacteria. However, this method involves considerable cost increases in the production process. But the activity of the acid proteinases could possibly be stopped by raising the pH of the ensilage to the basic range. At a ph of, for example 11 the ensilage would, from a microbial view-point, be preserved in the same way as

at a pH of 3.5-4.0. Ensiling by raising the pH directly with ammonia has previously been tried without success. However, with a basic ensilage it would be necessary to take into consideration the effect of neutral/basic proteinases. In neutral/basic conditions, these will contribute to the same splitting of proteins as the acid proteinases in acid conditions, and thereby a decline in the viscosity of the ensilage. We therefore decided to ensile industrial fish by first lowering the pH to 3.5 in order to irreversibly inactivate the neutral/basic proteinases. We then raised the pH to about 11 which inactivated the acid proteinases. We observed an ensilage of stable and durable consistency. Figure 5 shows the changes in viscosity as a function of the time elapsed after the start of ensiling during the above conditions. The viscosity decreased before the pH of the ensilage was adjusted upward. After increasing the pH of the ensilage, the decrease in viscosity was stopped, and the viscosity increased until it reached 50% of the viscosity recorded for ground industrial fish at the initial stage. It was assumed that the increase in viscosity could be attributed to the ability of unsplit protein chains to form gels during basic conditions. The increase in viscosity then ought to be dependent on the final pH of the ensilage, and on how long the acid treatment lasted before the pH was adjusted upward. When the acid treatment lasts too long, it is reasonable to assume that the protein chains are split

too much, and therefore will not gel sufficiently. Too short acid treatment could mean that the neutral/basic proteinases would not be inactivated, with the result that the viscosity increase obtained would not be stable. Figure 6 shows the viscosity increase as a function of time for various final pH values. The results show that it was necessary to adjust the pH up to 10.8 or more, in order to achieve the desired effect. Figure 7 shows the effect of acid treatment time on the capacity of the ensilage to form a stable gel with constant viscosity. The results show lower maximum viscosities for longer acid treatment times, probably due to decomposition of proteins. The acid ensilage, before the pH was adjusted to the basic range, had an activity of acid proteolytic enzymes corresponding to 60 µmol tyrosine equiv./mg min. This activity was stable as long as the pH was kept within the acid range. After pH had been adjusted to 11, no activity could be demonstrated either for acid or neutral/basic proteinases. The results so far show that the ground industrial fish can be preserved through ensiling with formic acid, followed by an adjustment of the pH to within the basic range. This method impedes the activity of the proteolytic enzymes, and the resulting paste does not have the liquid consistency of traditional ensilage. The ensilage was microbiologically stable and was stored at ambient temperature for 2 months. When this ensilage is used for fish feed, it is expected to require less addition of

binding agents. However, finished feed based on ensilage must have a pH within the neutral range. Therefore, it was necessary to demonstrate whether the gelling effect obtained in the basic range was reversible if the pH was adjusted toward neutral. Figure 8 shows that the basic ensilage to a large extent maintains its consistency in the pH range of 7 - 11. The small decrease in viscosity, observed when basic ensilage of a pH of 11 is titrated down to a pH of 7, is attributed to the liquefying factor of titration. The results indicate that the ensilage could be suitable as an ingredient in fish farm feed.

Table 1 shows the amino acid composition in a basic ensilage 25 hours after the start of ensiling, compared to the composition in an acid ensilage after the same time lapse. The ensilage technique itself does not appear to have affected the composition of amino acids to any significant extent. But no amino acid analyses were carried out for the basic ensilage after an extended storage period, so the analysis tells us nothing about the chemical stability of the amino acids during these conditions.

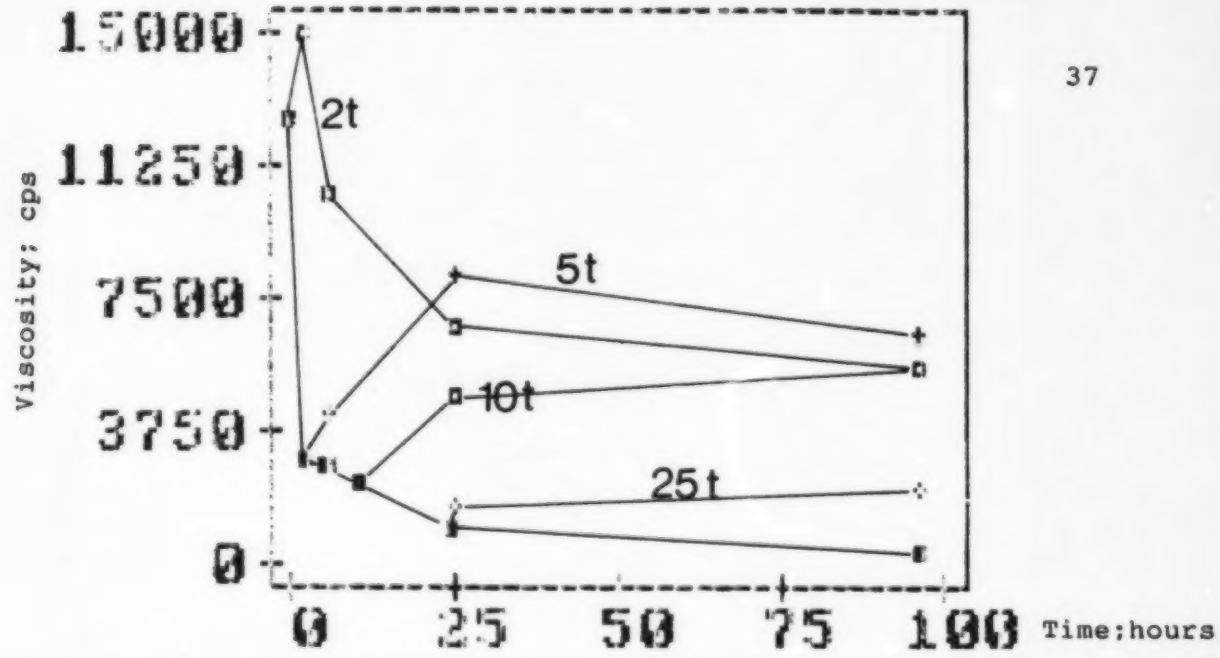

Figure 7. The figure shows viscosity (cps) as a function of time (hours) when pH in an acid ensilage of capelin (2% formic acid; 20°C) is adjusted upward to a pH of 10.7 (caustic soda) 2, 5, 10 and 25 hours respectively after start of ensiling.

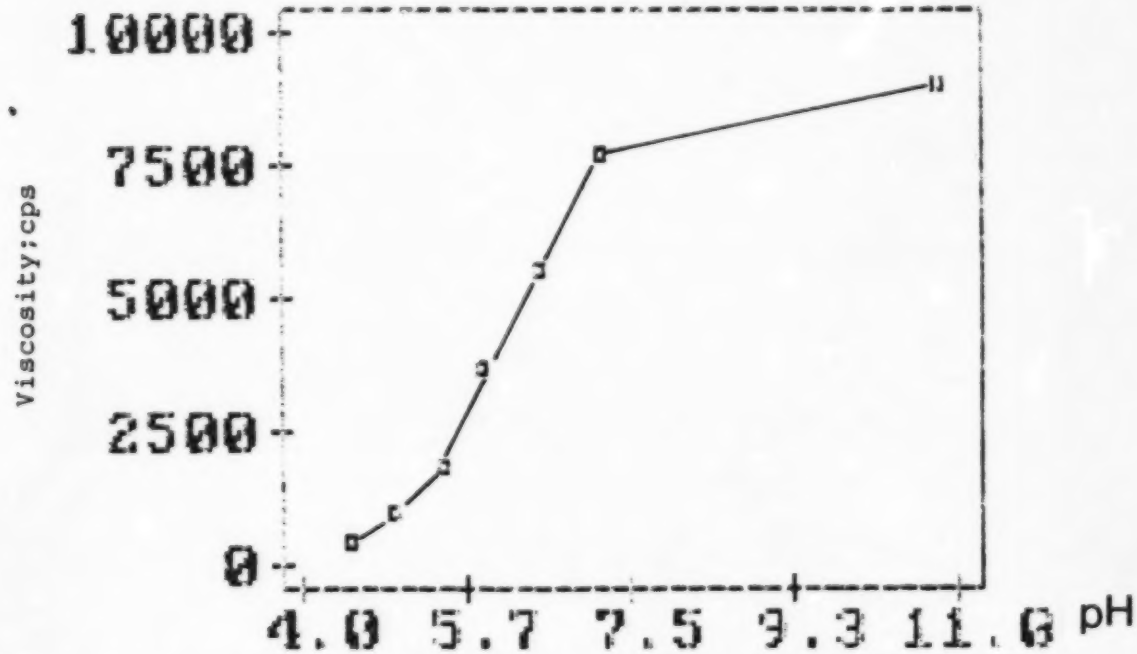

Figure 8. The figure shows viscosity in a basic ensilage (2% formic acid; 20°C, adjusted to pH 11 with caustic soda after 5 hours) as a function of the pH when titrated down to a pH of 4.0.

Amino acids	Amino acid composition (% of total)	
	Acid ensilage	Basic ensilage
Asparagine acid	2.62	2.83
Glutamine acid	6.54	6.44
Serine	3.64	2.90
Glycine	8.23	6.41
Histidine	2.01	2.40
Arginine	5.60	5.42
Threonine	5.32	4.93
Alanine	10.03	9.76
Proline	6.03	6.57
Ammonia	2.69	1.97
Tyrosine	4.05	3.46
Valine	9.48	10.42
Methionine	4.02	4.64
Cysteine	0.17	0.04
Isoleucine	5.28	6.57
Leucine	10.02	11.37
Phenylalanine	4.31	4.93
Lysine	9.89	8.47

Table 1. The table shows the composition of amino acids in acid and basic ensilages 25 hours after start of ensiling.

Basic ensilage produced according to the method described above, appears to satisfy the demands made on the consistency needed to manufacture an animal feed with acceptable texture. However, there does not appear to be an essential difference in the composition of amino acids of this ensilage and acid ensilage, but no studies have been done to clarify how the content of tryptophan and histidine is affected when stored for a long period of time. But it is worth noting that protein in a basic ensilage is decomposed only to a minor extent. The majority of the amino acids will

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be in protein bonds. This may contribute to improved amino acid stability in spite of the ensiling conditions.

In acid ensiling thiaminase will cause splitting of thiamine (vitamin B_1). The optimum pH of thiaminase is within the somewhat acid range (18), and will not have much affect on the content of thiamine in a basic ensilage. However, basic conditions will favour chemical decomposition of thiamine. Protein and amino acids are known to inhibit decomposition in acid conditions, while amino acids will hasten the decomposition of thiamine in basic conditions (2). According to what is already known about thiaminase and thiamine, this vitamin should be added to feed before it is given to animals. This also applies to ascorbic acid (vitamin C), which is also not stable when stored.

Studies were done to find out whether more of the collagen-containing structures in capelin dissolved during basic ensiling than during acid ensiling, and if this could help explain some of the observed gelling effect. A relatively high content of hydroxyproline is a characteristic of collagen. The soluble portion of acid and basic ensilage was hydrolyzed and analysed for hydroxyproline. It could not be demonstrated that there was more hydroxyproline in basic ensilage than in acid ensilage. This may be an indication

quantity of binding agents could be reduced by about 9% in a basic ensilage in relation to an acid ensilage, without any change in the consistency.

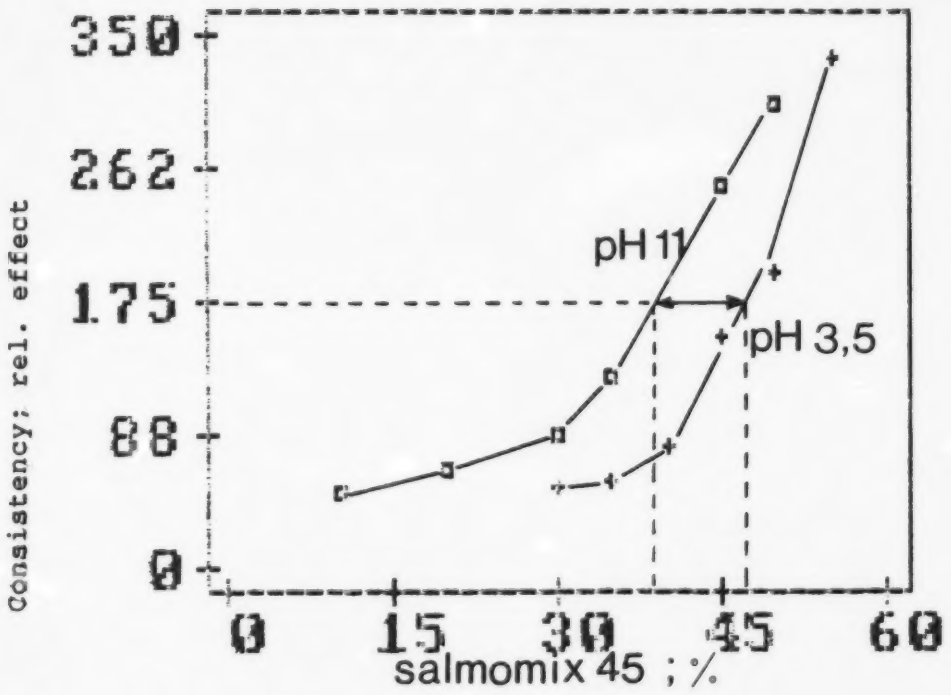

Figure 9. The figure shows consistency (rel. effect) as a function of added quantity of commercial binding agent (% Salminox 45) in acid and basic ensilage.

Trials were also carried out to compose a feed based on basic ensilage that was to contain small amounts of polysaccharides, have a consistency suitable for animal feeding and have a low solubility in water. Nutritionally, the feed was to be comparable to dry pellets. Table 2 shows the solubility of various feed mixes, compared to the solubility of one type of dry pellets.

Ensilage % Acid	Basic	Capelin meal NSM(%)	Binding agent	Solubility (mg/ml) 4 min. ambient temp.
	64.3	25.7	10% Salmomix 10	7.1
	60.0		40% Salmomix 45	5.7
	70.0	28.0	2% Guar gum	2.5
	70.0	28.0	2% Guar gum + 0.5% Xanthan	2.7
50.0			50% Salmomix 45	7.9
60.7		24.3	15% Salmomix 10	10.3
	45.0	55.0		31.6
	47.0	50.0	3% Drinde	24.3
	40.0	60.0		11.3
	54.0	45.0	1% Guar gum	6.6
	53.0	45.0	1% Guar gum + 1% Protatek	6.1
Dry feed Salmonix Edel				2.1

Table 2. The table shows the composition (%) of animal feed based on acid and basic ensilage, and the solubility of the feed in water (mg dry matter/ml)

Table 2 indicates that a basic ensilage can be used to produce an animal feed consisting of ensilage, capelin meal

and only 1% polysaccharides (guar gum). Guar gum is completely indigestible. It will pass through the fish's intestines without breaking down, and the result is that sugar cannot be absorbed. The solubility of this feed is acceptable, similar to that of dry pellets. A similar feed cannot be produced from acid ensilage.

On the basis of the results obtained, it was decided that the feed trials were to be carried out using a feed with the composition shown in Table 3.

basic ensilage	55% (w/w)
capelin meal (NSM)	43% (w/w)
guar gum	1% (w/w)
vitamin meal (Skretting)	1% (w/w)

Table 3. The table shows the composition (%) of the feed made from basic ensilage that was used in the feed trials.

The nutritional content of the feed is shown in % of wet weight in Table 4.

* protein	36.8 % *
* fat	16.0 % *
* carbohydrates	1.5 % *
* ash	5.4 % *
* water	38.5 % *

Table 4. The table shows the nutritional composition of the feed used in the trials (%).

With this feed the fish will get 52.8% of its energy supply from protein and 47.2% from fat. The feed will have an energy content of 18.47 MJ/kg dry matter. In comparison, the energy content of dry pellets (Tess Edel) is 18.53 MJ/kg dry matter. The feed thus appears to be balanced, and it should be possible to compare the growth of fish fed with dry pellets and with feed made from basic ensilage. When preparing the finished ensilage feed on a daily basis from a large batch of basic ensilage, the addition of vitamin meal will ensure correct supply of vitamins in the diet.

Basic ensilage for animal feed was produced on a large scale as described in "Material and methods". To the ensilage was added 200 ppm ethoxyquine as an antioxidant. Peroxide numbers were measured in the finished ensilage in order to make sure that the ensilage had not gone rancid, which would decrease the appetite of the trial fish.

Feed trials

The feed trials were carried out at FTFI's[1] fish farm outside Tromsø. Table 5 shows the number of fish, distribution into nets, weight and length at the start of the feed trials. An analysis of variance showed that there was no statistical difference in weight and length between the 4 trial groups. It is worth noting that the biological material, coupled with standardized procedures for weighing, results in high standard deviations for weight and length measurements.

Net	No. of fish	Mean weight g	Stand. deviat.	Mean length mm	Stand. dev.
A	100	229.4	57.6	290	23.3
B	99	228.8	59.3	294	22.5
C	103	213.5	42.5	287	21.8
D	100	225.8	61.2	300	27.3

Table 5. The table shows fish weight, length and numbers at the start of the feed trials.

Fish in net A and B were fed dry feed (Tess Edel; Skretting), while fish in net C and D were fed feed based on basic ensilage as described above. The fish were taken from the same source and distributed randomly into the 4 nets.

[1] Transl. note: Research Institute for Fisheries Technology

The nets were chosen randomly and nets B and D bordered on the edge of the fish farm, while nets A and C were located in the centre of the fish farm. Weight and length were based on weighing and measuring of 20% of the population. The ensilage feed was prepared daily from a larger batch of basic ensilage. Feeding was carried out manually and growth and mortality were recorded. It was observed that the fish ate the ensilage feed with a good appetite.

Growth and increase in length were measured 6.5 and 13 weeks after the start of the trials. The fish were weighed and measured only 3 times in consideration of the negative effects of stress and anaesthesia. Table 6 shows weight increase during the feeding period. Mean weight increase per fish was approximately 290 g.

Net	Feed type	Weight (g) 6.5 weeks	Stand. dev.	Weight (g) 13 weeks	Stand. dev.
A	Tess Edel	280.9	96.1	525.4	148.8
B	Tess Edel	303.6	83.8	573.0	154.9
C	Ensilage	307.8	78.3	527.8	142.7
D	Ensilage	287.5	64.5	431.0	91.4

Table 6. The table shows growth (g) during the trial period and feed type for the different trial groups.

An analysis of variance showed that there was no difference in weight increase between fish from nets A, B

and C. Fish in net D grew less, which concurred with the observation that these fish had a lower appetite. No reasonable explanation was found for this. However, it may be the result of the trials having taken place in an outdoor fish farm, and not in an enclosed system were all parameters can be better controlled.

Table 7 shows growth increase (mm) during the feeding period. The length of the fish increased by an average of approximately 80 mm during the period.

Net	Feed type	Length (mm) 6.5 weeks	Stand. dev.	Length (mm) 13 weeks	Stand. dev.
A	Tess Edel	315	35.4	372	30.7
B	Tess Edel	320	27.8	380	34.1
C	Ensilage	325	27.4	382	33.4
D	Ensilage	317	23.6	363	26.8

Table 7. The table shows trial fish growth in mm during the feeding period, and feed type.

Due to technical problems, during the first test period the fish were fed for short periods with feed that differed from that specified in this report. This feed contained in part larger amounts of polysaccharides. Therefore, the feed factor calculations were based exclusively on data from the last feeding period (the last 6.5 weeks). Fish growth in this period, total quantity dry matter given in the period,

the period, total energy content of the feed and its theoretical content of energy provided by polysaccharides are shown in Table 8. The digestibility of the polysaccharide portion was not taken into account. It is worth noting that the energy contribution from polysaccharides in dry pellets constitutes 6.5%, while the energy contribution from polysaccharides in ensilage-based feed constitutes 0.87%. Moreover, most of the polysaccharides in the ensilage feed consist of indigestible guar gum.

Net	Total growth in the period (gram)	Dry matter supplied (gram)	Energy supplied through feed (KJ)	
			total	from polysacch.
A	23924.6	19300.3	336951.8	21793.8
B	26670.6	18457.3	322234.4	20842.0
C	22665.7	16006.3	299074.9	2615.0
D	14325.0	14015.3	261872.4	2284.7

Table 8. The table shows total growth in the various nets (g) during the last half of the trial period, dry matter (g) fed during the same period, total energy contribution (KJ) and the theoretical energy contribution from polysaccharides (KJ).

Table 8 shows good growth in relation to the amount of feed given, both for feed based on basic ensilage (nets C and D) and dry feed (nets A and B). The feed factor is shown in Table 9, with corrections for the difference in water content between the two types of feed. It is recorded as

supplied dry matter/growth. The calculations were done with 14% water in dry pellets, and 38.5% water in ensilage feed.

Net	Feed type	Feed factor	Supplied dry matter per growth
A	Tess Edel	0.92	0.81
B	Tess Edel	0.79	0.69
C	Ensilage	0.98	0.71
D	Ensilage	1.36	0.98

Table 9. The table shows feed factor and supplied dry matter/growth for fish in the different nets during the last half of the trial period.

The results of the feed trials show that there is no statistical difference in the growth of fish in nets A, B and C. The fish in net C utilized the ensilage feed in the same way as dry pellets. The fish in net D utilized the ensilage feed somewhat less. The feed factor is relatively low in all the trial groups. A feed factor as low as around 0.7 approaches the theoretically possible. A lower feed factor than what is traditionally observed in fish farming was attributed to the fact that feeding was done manually, with minimal wastage. Neither can it be ruled out that water collected with the anaesthetized fish during weighing may have resulted in somewhat high weight recordings. This will also show up as low feed factors.

By making corrections for the water content of the feed, the supplied dry matter/growth for ensilage feed and dry pellets can be directly compared. The results show that fish fed with feed made from ensilage in net C utilized the feed as well as fish fed with dry pellets in net A and B.

Mortality during feeding in the trial period was low. Only one dead fish was observed during the trial period.

7. CONCLUSION

The enzymatic decomposition processes during acid ensiling of industrial fish (2% formic acid; 20°C) were halted by increasing the pH to 11 with caustic soda. The basic ensilage was microbially stable and had a consistency suitable for use as animal feed without any large amounts of binding agents. Feed trials were carried out with the following fish feed:

55% basic ensilage
43% capelin meal
1% guar gum
1% vitamin meal

The feed trials indicated that the feed based on ensilage resulted in good growth and acceptable feed factor values.

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